

EARTHWORM COELOMOCYTE DERIVED CYTOTOXICITY IS NOT RESCUED BY ANTI-LYSENIN PRETREATMENT

ENGELMANN PÉTER¹, SOMOGYI ILDIK², MÁCSIK LEVENTE LÁSZLÓ¹,
POLLÁK EDIT², MOLNÁR LÁSZLÓ², NÉMETH PÉTER¹

¹Department of Immunology and Biotechnology,
Clinical Center, University of Pécs, Pécs, Hungary

²Department of Comparative Anatomy and Developmental
Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary

Objectives: Coelomocyte subpopulations have essential role in earthworm cellular immunity. In addition to secreted antimicrobial factors of earthworms, there is a firm evidence for certain cytotoxic components. Previously, we observed rapid cell lysis of multiple tumor cell lines caused by coelomocyte lysate or supernatant of *in vitro* cultured coelomocytes. Moreover, we demonstrated that coelomocyte lysate induced a caspase independent apoptotic-like cell death in tumor (Sp2 and HeLa) cell targets using different technical approaches (transmission electron microscopy, TUNEL assay, Annexin V staining, assessment of mitochondrial membrane potential changes, and measurement of Ca²⁺ influx).

Methods: Our recent aim was to study whether a lytic protein named as lysenin participates in the observed cytotoxic mechanisms. For this attempt we applied our in-house developed anti-lysenin monoclonal antibody (a-EFCC5) to inhibit the coelomocyte lysate evoked toxicity in various assays (TUNEL, Annexin V, MMP, Ca²⁺ influx).

Results: Indeed, anti-EFCC5 pre-treated coelomocyte lysate caused less TUNEL positivity in HeLa target cells; however there was detectable DNA fragmentation compared to negative controls. Similarly, pretreatment with a-EFCC5 mAb rescued Sp2 target cells from the loss of mitochondrial membrane potential, but it was still significantly different from the control cells. A-EFCC5 pretreated coelomocyte lysate induced an attenuated phosphatidyl-serine translocation of target cells revealed by Annexin V/propidium iodide staining. In the case of Ca²⁺ kinetics, mAb pretreatment caused a fall in lysate-evoked Ca²⁺ influx compared to the untreated lysates, but it was clearly noticeable in contrast to the negative controls.

Conclusion: Anti-lysenin specific mAb pretreatment rescued the majority but not all target cells from coelomocyte-induced death. These data suggest that factors other than lysenin may also participate in the cytotoxic activity of coelomocytes. Our observations underscore the complexity of cytotoxicity-related immune response in the earthworm.

STUDYING THE NEGATIVE REGULATORY FACTORS OF THE PROPIONIBACTERIUM ACNES-INDUCED SIGNALING PATHWAYS IN IN VITRO CULTURED IMMORTALIZED KERATINOCYTES

ERDEI LILLA¹, GÁBOR TAX¹, SZILVIA BEÁTA BOLLA¹, EDIT URBÁN³,
LAJOS KEMÉNY^{1, 2}, KORNÉLIA SZABÓ²

¹Department of Dermatology and Allergology,
University of Szeged, Hungary

²MTA-SZTE Dermatological Research Group, Szeged, Hungary

³Institute of Clinical Microbiology, University of Szeged,
Hungary

Propionibacterium acnes (*P. acnes*) is a resident microbe of the healthy human skin microbiome, but also know to simulate immune and inflammatory events via the activation of Toll-like receptors (TLRs). These molecular pathways are well characterized, but little is known about the endogenous negative regulatory mechanisms in the keratinocytes that counteract the bacterium-induced signaling events and thus may protect the host from the prolonged, uncontrolled and often destructive inflammation.

In our studies we aimed to characterize the keratinocyte expression of endogenous negative regulators of TLR signaling pathways previously identified in other cell types, and to analyze their possible role in the attenuation of the *P. acnes*-induced molecular events. For that, we studied the basal mRNA and protein expression of selected genes (SIGIRR, TOLLIP, TNFAIP3, TNIP1) in a human, *in vitro* cultured immortalized keratinocyte cell line (HPV-KER) by real time RT-PCR and western blot analysis.

Our results suggest that all the investigated negative regulators are expressed in HPV-KER cells and the TNFAIP3 and TNIP1 mRNA expressions significantly and dose dependently increase in response to the bacterium. At the protein level, we found increased TNFAIP3 and decreased SIGIRR expressions following the bacterial treatment, and these events also appeared to be dose dependent. Next, we compared the effect of two *P. acnes* strains (889, 6609) belonging to different phylogenetic groups within the species (IA and IB, respectively), but no major differences have been observed in the induced expression changes.

Our study suggests that in our *in vitro* model system *P. acnes* causes a dose-dependent activation of downstream TLR signaling processes. However, parallel to that specialized, endogenous negative regulators are also expressed in these cells, which may control the bacterial-induced molecular events, and thus can be important for the maintenance of epidermal homeostasis.